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Nanoindentation of human donor cornea for detecting the effectiveness of laser-induced collagen crosslinking

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One of the most important characteristics of a material is its mechanical properties. Methods to determine the micromechanical properties of biological samples are highly varied. At the moment, to measure the local mechanical characteristics of samples, the nanoindentation method has been gaining popularity. The method is based on indenting a sample with a spherical tip attached to a flexible cantilever and detecting the cantilever's deflection interferometrically, which results in a load-indentation curve. This curve is used to calculate the Young's modulus E. This method allows one to work with various biological materials, as well as to measure the local E of the sample surface. In our research, we use a PIUMA nanoindenter (Optics11) to estimate the effectiveness of a method developed for hardening the human cornea.

One of the most common diseases of the cornea is keratoconus, a pathological process leading to thinning of the cornea and, as a result, loss or deterioration of vision. The traditional approach to the treatment of keratoconus involves UV-photocrosslinking of the corneal collagen with riboflavin as a photoinitiator. However, such a method has disadvantages, such as difficulty of a precise regulation of cornea irradiation and, as a consequence, damage to the healthy regions and impossibility of crosslinking fibers through the whole thickness. Therefore, for the effective treatment of keratoconus without negative consequences, a two-photon laser femtosecond crosslinking (2P-CXL) method at the wavelength of 525 nm was developed. The most important advantage of 2P-CXL is its extremely local effect: 0.5 - 5 μm along the X and Y axes (the width of the laser radiation caustic), 2 - 20 μm along the Z axis (the caustic length) which allows collagen to be crosslinked without damaging the endothelial and epithelial corneal layers.

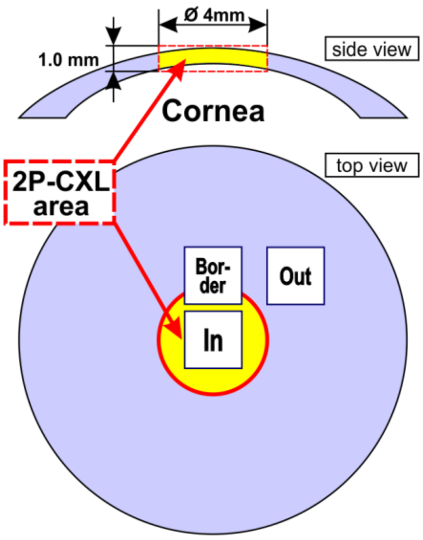

Figure 1. Schematic image of the cornea with the designated areas in which the femtosecond treatment and measuring the effective Young's modulus by nanoindentation took place. Yellow (IN) is the region where the cornea was treated with 2P-CXL. Blue (OUT) is the intact cornea region. Nanoindentation was performed inside both the regions and also at the border (BORDER) between the regions.

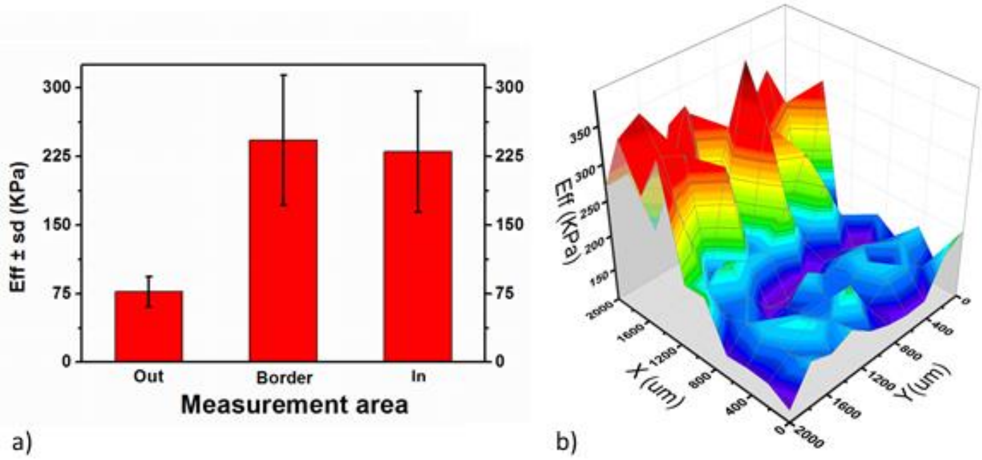

Figure 2. (a) The surface effective Young's moduli Eff for the three corneal areas, in accordance with Figure 1; (b) E_{ff} distribution over the corneal surface at the border of the treatment zone.

The sample without epithelium was treated with 0.1% riboflavin solution for 30 minutes prior to the laser irradiation, and in the course of the experiment the treatment was repeated every 2 minutes using a pipette. The treatment of the human donor cornea with 2P-CXL was performed layer by layer in the central area with a diameter of 4 mm and a thickness of 1mm (Fig. 1). The local mechanical characteristics of the cornea were determined with a PIUMA nanoindenter using the Hertzian contact mechanics model for a spherical body indenting a flat surface.

The E measurements were carried out in a solution for the cornea storage heated to 34°C, which is a normal temperature for the human cornea. For indentation we used a cantilever with a spring constant of 2.94 N/m and a tip with the 45 μm radius of curvature. The samples were immobilized at the bottom of a Petri dish using a weight. The area of the E mapping was 2000×2000 μm with the step of 200 μm by the X- and Y-axes.

In Figure 2a, a histogram of the effective Young's moduli E_{ff} of the corneal surface is displayed for the three regions of measurements according to Figure 1. It demonstrates that the E_{ff} value at the border of the treatment and within the area of the femtosecond treatment grows by about three times (Fig. 2a), from 77±16 kPa to 230±66 kPa. In Figure 2b, a typical E_{ff} 3D plot is depicted, located in the region of the border between the treated and intact zones.

The increase of the Young's modulus of the human donor cornea, which was determined by nanoindentation, allows us to make a conclusion about a good potential of a femtosecond laser with a wavelength of 525 nm for the clinical use, to increase the stiffness of the cornea by 2P-CXL.

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